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Rapid Test for Urease and Phenylalanine Deaminase Production

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A rapid urea-phenylalanine medium was effective for the identification of *Proteus* and, with one exception, *Providencia*. Most *Klebsiella* and a few *Enterobacter were* urease-positive with this method.

In 1968 Vassiliadis and Politi (3) devised a combined medium for the detection of urease and phenylalanine deaminase production. The medium they formulated required 24 hr of incubation, but it did have the advantage that two tests could be performed in one tube. We decided to adapt this combined urease-phenylalanine deaminase medium for use as a rapid test. The medium was made by dissolving 1 g of yeast extract (Difco), 2.0 g of $(NH_4)_2SO_4$, 3.0 g of NaCl, 1.2 g of K_2HPO_4, 0.8 g of KH_2PO_4, and 2.5 g of 1-phenylalanine or 5.0 g of DL-phenylalanine (Nutritional Biochemicals) in 997 ml of deionized water, with heat if necessary. After cooling, 5 g of urea (Difco) and 3.5 ml of phenol red solution (0.5 g of phenol red, 20 ml of 0.1 N NaOH, and 230 ml of deionized water) were added. The final *p*H was 6.8; no adjustment was necessary. The complete medium was sterilized by filtration and dispensed in 0.3-ml portions into test tubes (13 by 100 mm) and capped. The tubes were stored at −20 C until used. The tubes were thawed before inoculating, and a large loopful of growth from triple sugar-iron-agar was emulsified in the medium. The tubes were incubated for 1, 2, 3, and 4 hr at 35 C, and the production of urease and phenylalanine deaminase was determined. Organisms which were urease-positive produced a deep pink color. Faint pink colors were called negative. After reading the urease reaction, one or two drops of 1% HCl were added to obtain an acid *p*H (yellow). Then two drops of 10% $FeCl_3$ were added. Any green color produced within 10 sec constituted a positive test for phenylpyruvic acid. Negative reactions were yellow.

We tested 254 species of *Proteus*. All of the 104 strains of *P. mirabilis* and 45 strains of *P. vulgaris* were urease-positive in 1 hr; 50 of 51 strains of *P. morgani* were urease-positive in 1 hr, the one other strain becoming positive in 2 hr; 44 of 54 strains of *P. rettgeri* were urease-positive in 1 hr, the other 10 strains becoming positive in 2 hr. All of these organisms were positive for phenylalanine deaminase in 1 hr.

Twenty-five strains of *Providencia* tested were urease-negative. Twenty-four of these strains were phenylalanine deaminase-positive within 1 hr. The one negative strain was positive with phenylalanine agar (Difco).

Other genera of the family *Enterobacteriaceae* tested included 4 strains of *Citrobacter*, 4 strains of *Serratia*, 2 strains of *Arizona*, 4 strains of *Salmonella*, 3 strains of *Shigella*, 1 strain each of enteropathogenic *Escherichia coli* and *Edwardsiella tarda*, 605 strains of *E. coli*, 108 strains of *Enterobacter*, and 284 strains of *Klebsiella*. None of these 1,016 strains was positive for phenylpyruvic acid. Eighty seven per cent of the *Klebsiella* were urease-positive within 4 hr. Ninety per cent of the *Enterobacter* were urease-negative. All other organisms listed were urease-negative.

The efficacy of the urea-phenylalanine rapid test medium appears clear. It fulfills diagnostic needs in that it is accurate and rapid and two tests can be performed in one tube. The introduction of this medium into routine identification schemes may facilitate the recognition of lactose-positive *Proteus* and *Providencia* species (2) as well as to reveal the more frequent incidence of *Yersinia* (1) infections.

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